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(54) Abstract Title
Cleaning compositions containing enzymes

(57) Enzyme-containing aqueous cleaning compositions having extended stability at high temperatures comprise: an enzyme system comprising a protease and an amylase; a boron-containing compound; a polyhydroxy-containing compound; an alkanolamine; a water-soluble calcium salt; a nonionic surfactant; an alkanol; and optionally, a brass corrosion inhibitor. Particularly intended for cleaning of medical equipment.

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IMPROVEMENTS IN OR RELATING TO ORGANIC COMPOSITIONS

The present invention relates to cleaning compositions which are particularly useful for the cleaning of medical instruments and related items, especially those cleaning compositions including enzymes.

Reliable cleaning of medical instruments is crucial to the efficient operation of hospitals and health care facilities in general. Many approaches have been taken to provide the medical community with a suitable cleaner for instruments and surfaces which have come in contact with organic materials (proteins, fats, blood, carbohydrates, membranes and similar materials) and need to be cleaned before another use.

Enzyme-containing cleaning compositions have been viewed as a satisfactory, if not completely ideal, solution to this problem. Enzyme-containing cleaning compositions are very heat sensitive. This is because enzymes tend to degrade rapidly at the temperature rises above about 37°C. Even using enzyme stabilizers, an enzyme quickly loses from 20% to about 30%, or even more, of its activity within a month if stored at 40°C.

Typical prior art enzyme-containing aqueous cleaning compositions also cause rust stains to steel surfaces and components. This can be a significant drawback for a hospital environment.

There is a distinct need in the art for an enzyme-containing aqueous cleaning composition with high stability towards storage at elevated temperature, so that the composition retains a significant degree of its enzymatic activity after storage under such conditions, as compared to its initial enzymatic activity.

The invention provides an aqueous cleaning composition containing enzymes. This composition is designed to be useful in the cleaning of medical instruments. The surprising benefit of the present invention is the discovery that the composition is stable at elevated temperatures. Certain embodiments of the composition surprisingly do not cause excessive rusting of steel, thus permitting in some cases such compositions to be formulated without the addition of a steel corrosion inhibitor.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

The enzyme-containing aqueous cleaning compositions of the invention comprises an enzyme system which acts to assist in the degradation and removal of matter from the objects to be cleaned.

The enzyme system includes a protease. Any proteases which are effective in breaking down proteins, particularly animal-derived proteins, may be used in the compositions according to the invention. Useful proteases are derived from non-pathogenic, alkalophilic strains of bacteria, and in particular from the genus *Bacillus*. This genus includes *Bacillus subtilis*, *Bacillus lentus*, and *Bacillus licheniformis*. These are referred to as alkaline proteases. Proteases which can be derived from sources other than those elucidated above can be used.

The activities of proteases are dependent on their source, that is, the particular bacteria, the purity and assay conditions. For practical reasons, the activity of the enzyme is often expressed in preference to the weight of a sample containing the enzyme. The enzyme activity of proteases is described in units which relate to the method of enzyme analysis used. The units include Glycine Units (GU), Delft Units (DU), Showa Denko Protease Units (PU), casein digestion units (CDU), Showa Denko Units (SU), Kilo Novo Units (KN) and Anson Units (AU).

Desirably, the protease constituent exhibits an activity of at least about 0.10 Kilo Novo protease units/gram, desirably at least 0.30 KNP units/gram, and still more desirably the protease constituent exhibits an activity of at least about 0.48 KNP units/gram. These protease units are known to the art and are determinable by well-

known techniques, and may be obtained from Novo Nordisk (Copenhagen, Denmark). The proteases further desirably exhibit activity in the pH range of about 3.5 to about 13.0, but preferably exhibit activity in the pH range of about 7.0 to about 10.5.

Various commercially available protease-containing preparations are available, such as ALKAPRO available from the Geo. A. Jeffreys & Co., Inc. (Salem, Virginia), described as being an alkaline serine-type protease from a bacterial origin, which exhibits an enzyme activity level of at least about 400,000 CDU protease units/gram which is useful in the pH range of from about 3.5 to about 13.0, and which exhibits optimal activity at a pH in the range of about 7.0 to about 10.5. Another useful protease is commercially available under the trade name SAVINASE, including SAVINASE 16.0 L (ex. Novo Nordisk). Other useful proteases include ALCALASE 2.5 L, or DURAZYME 16.0 L (ex. Novo Nordisk).

The enzyme-containing aqueous cleaning compositions of the invention further comprise an amylase. Amylases which are useful in the compositions according to the invention are those which are effective in breaking down complex polysaccharides and starches into simpler units, including sugars. Such useful amylases include those which are referred to as alpha-amylases, beta-amylases, iso-amylases, pullulanases, maltogenic amylases, amyloglucosidases, and glucoamylases, as well as other amylase enzymes not particularly elucidated here. These include endo- and exo-active amylases. Useful amylases can be obtained from a wide variety of sources, including microorganisms of the genus *Bacillus*. By way of non-limiting example, specific microorganisms include: *Bacillus subtillis*, *Bacillus amyloliquefaciens*, *Bacillus stearothermophilus*, *Bacillus licheniformis* especially containing a *Bacillus sterothermophilus* gene for alpha-amylase, *Bacillus subtilis* containing a *Bacillus megaterium* gene for alpha-amylase, as well as *Bacillus acidopullulyticus*. Other sources include for example, barley malt, certain animal pancreatic tissue as well as others not elucidated here but which are nonetheless known to the art.

The activity of amylases may be described in units of bacterial amylase units/gram according to known methods such as those disclosed in the Food Chemicals Codex, as well as by other art-recognized analytical techniques. Useful amylase-containing preparations contain at least about 0.5 Kilo Novo units/gram (KN

units/g), more desirably at least about 1.5 KN units/g, and most desirably at least about 3 KN units/g.. Such Kilo Novo units/gram are known to the art, are determinable by well-known techniques, and may be obtained from Novo Nordisk (Copenhagen, Denmark).

Further useful amylase-containing preparations are available from a variety of commercial sources including for example, a product marketed as IC 24,000 by the Geo. A. Jeffreys & Co., Inc. (Salem, Virginia), described as being an amylase/carbohydrase preparation from a bacterial origin, which exhibits an enzyme activity level of at least about 24,000 bacterial amylase units per gram. Another useful amylase is commercially available as TERMAMYL 300L (ex. Novo Nordisk).

Optionally but desirably, the enzyme system of the compositions according to the invention can further comprise amounts of further secondary enzymes, including but not limited to lipases, cellulases, pectinases, carbohydrases, beta-glucanases, hemicellulases, and xylanases. The addition of such further secondary enzymes to the compositions according to the invention aids in the cleaning of objects placed in these compositions.

The enzyme-containing aqueous cleaning compositions of the invention further comprise a boron-containing compound. Boron-containing compounds are known to stabilize certain enzymes, including protease enzymes. Although the claims are not limited by any particular theory regarding the function of the boron-containing compound, it is believed that boron-containing compounds are able to stabilize the enzymes by binding in a particular region of the enzymes.

Suitable boron-containing compounds include the boronic acids of structure $RB(OH)_2$, in which R is a short chain alkyl group such as methyl, ethyl, propyl or butyl, or R can be an aryl group. R can be substituted with hydroxyl, amino, or carboxyl groups as desired to modify the solubility of the boron-containing compound, which should be highly soluble in water. Also useful is boric acid, $B(OH)_3$. Polyborates which can be formed *in situ* or added as a species can also be used in the compositions according to the invention. Such polyborates include species having molecular formulas of $B_3O_3(OH)_4^-$, $B_3O_3(OH)_5^{2-}$, $B_5O_6(OH)_4^-$, $B_4O_5(OH)_4^{2-}$, as well as the borate $B(OH)_4^-$. Such species are generally present as their alkali metal

salts. Also included are hydrated forms of alkali metal borates, such as Borax. Boron oxides are also useful in the compositions according to the invention.

Preferred boron-containing compounds are boric acid, alkali metal borates, and boronic acids. The boron-containing compound is desirably present in an amount of from about 0.2% to about 10% by weight, or more desirably, from about 2% to about 5% by weight of the composition.

The enzyme-containing aqueous cleaning compositions of the invention further comprise a polyhydroxy-containing compound. This class of constituent is believed to aid in stabilization of enzymes by sequestering water molecules which otherwise tend to lead to enzyme destabilization.

Useful polyhydroxy-containing compounds are those in the class of alkylene glycols such as ethylene glycol, propylene glycol, as well as glycerine. Preferred polyhydroxy-containing compounds are propylene glycol and glycerine. These materials are widely available from general chemical suppliers such as Aldrich Chemical Co. (St. Louis, Missouri). The polyhydroxy-containing compound is desirably present in an amount of from about 10% to about 30% by weight, or more desirably, from about 10% to about 20% by weight of the composition.

The enzyme-containing aqueous cleaning compositions of the invention further comprise an alkanolamine. The alkanolamine is useful in the compositions according to the invention for two reasons: to adjust the pH of the material, and to provide an enhanced cleaning benefit.

Useful alkanolamines in the inventive compositions include monoalkanolamines, dialkanolamines, trialkanolamines, and alkylalkanolamines such as alkyl-dialkanolamines, and dialkyl-monoalkanolamines. The alkanol and alkyl groups are generally short to medium chain length, that is, from 1 to 7 carbons in length. For di- and trialkanolamines and dialkyl-monoalkanolamines, these groups can be combined on the same amine to produce for example, methylethylhydroxypropylhydroxylamine. One of skill can readily ascertain other members of this group. Preferred alkanolamines are trialkanolamines, including triethanolamine. The alkanolamine is desirably present in an amount of from about 1% to about 10% by weight, more desirably from about 3% to about 5% by weight of the composition.

The enzyme-containing aqueous cleaning compositions of the invention further comprise calcium ion. The presence of calcium ions in the compositions is believed to enhance the stability of the enzymes present. The calcium ions are desirably present as water-soluble salts, such as the halide salts, including calcium chloride. The calcium ion is desirably present in an amount of at least about 0.01%wt., but less than about 2.0% by weight, more desirably less than about 1.0% by weight of the composition. The calcium ion is advantageously included in amounts of at least about 0.05%wt.

The enzyme-containing aqueous cleaning compositions of the invention further comprise a nonionic surfactant. It has been found that the nonionic surfactants advantageously assist in cleaning, providing enhanced cleaning effectiveness.

By way of non-limiting example, useful nonionic surfactants include those with hydroxyl, ether, amine oxide, phosphine oxide, sulphoxide, propargyl, ester, or amide functionalities. Among these, ethoxylates are considered especially useful, and these include alcohol ethoxylates, mono alkanolamide ethoxylates, fatty amine ethoxylates, fatty acid ethoxylates, ethylene oxide/propylene oxide copolymers, and alkyl phenol ethoxylates. Further exemplary useful nonionic surfactants include those having multiple hydroxyl molecules such as glucosides, glycerides, glycol esters, glycerol esters, polyglycerol esters and polyglycerides, polyglycosides, sorbitan esters and sorbitan ester ethoxylates, and sucrose esters.

Desirably, the one or more nonionic surfactants are those which are known to have reduced foaming characteristics, as excessive foaming of the inventive compositions may reduce the overall cleaning efficacy of the compositions. Excessive foaming is desirably avoided, especially where the present compositions are intended to be utilized in machinery or devices which aid in, or which perform in the cleaning of instruments, etc.

Preferred nonionic surfactants are the ethylene oxide/propylene oxide copolymers. There are many possible variations within this class, among the most useful being of three types: (1) the PLURONIC copolymers having the formulas (EO)n(PO)m(EO)n, where EO is ethylene oxide, PO is propylene oxide, n is an integer from 1 to about 20,000, and m is an integer from 1 to about 20,000; (2) the

reverse PLURONIC copolymers having the formulas (PO)n(EO)m(PO)n, with the same abbreviations as above; and (3) the EO/PO copolymers with alkyl end group on one or both ends. The copolymers above are represented as block copolymers, although random copolymers are also useful in the compositions according to the invention. It should be noted that even those copolymers commercialized as block copolymers will have some degree of randomness. Glycerol-based EO/PO block and random copolymers and ethylene diamine-based block and random copolymers are also useful in the compositions according to the invention. Useful information on nonionic surfactants is found, for example, in The Handbook of Surfactants, M.R. Porter, (Blackie Academic and Professional, 1994, London.)

Particularly preferred are the EO/PO block copolymers commercially available as PLURONIC surfactants (ex. BASF). The nonionic surfactant is desirably present in an amount of from about 1% to about 20% by weight, more desirably from about 2.5% to about 10% by weight, and most desirably from about 5% to about 10%wt. based on the total weight of the composition of which it forms a part.

The enzyme-containing aqueous cleaning compositions of the invention further comprise an alkanol. The alkanol is useful for preventing excessive foaming, and for providing an antimicrobial effect to the compositions.

Useful alkanols include short chain alcohols, such as C_1-C_8 primary, secondary and tertiary alcohols, e.g., methanol, ethanol, n-propanol, isopropanol, and butanol. Particularly preferred alkanols include the isomers of C_3 alcohols, particularly isopropanol. C_2-C_8 diols may also be used in the alkanol constituent. The alkanol is desirably present in an amount of from about 3% to about 10% by weight, more desirably from about 5% to about 10% by weight of the composition.

The enzyme-containing aqueous cleaning compositions of the invention further comprise water sufficient to provide the remaining weight of the composition. Deionized water is preferably used.

The enzyme-containing aqueous cleaning composition desirably has a pH of from about 7.0 to about 8.0, more desirably from about 7.3 to about 7.7.

According to particularly preferred embodiments, the inventive compositions retain a significant proportion of their initial enzymatic activity upon long term storage at elevated temperatures. According to certain particularly preferred

embodiments, the inventive compositions retain at least 50%, more desirably at least 60%, more preferably at least 70%, still more preferably at least 75% of their initial enzymatic activity as compared to their initial enzymatic activity, following storage of 7 days at 40°C. Particularly preferred embodiments exhibit such retention of initial enzymatic activity subsequent to 30 days, more desirably 45 days, and most desirably subsequent to 60 days of storage at 40°C. Still more preferred embodiments may exhibit still further improvements in enzymatic stability.

The inventive compositions may include one or more further optional constituents. These include, inter alia, compounds which aid in reducing metal corrosion, as well as others which improve the aesthetic appeal of the compositions. For example, materials which aid in reducing brass corrosion are particularly useful for preventing corrosion damage of any brass components in the objects to be cleaned by the compositions according to the invention. A typically useful brass corrosion inhibitor is a triazole compound, such as tolyltriazole, an alkali metal salt of tolyltriazole, benzotriazole, carboxybenzotriazole, or blended products. These compounds are commercially available as COBRATEC TT100, COBRATEC TT-50-S, COBRATEC CBT, COBRATEC CBT-E, COBRATEC 99, COBRATEC 928, COBRATEC 911S (PMC Specialties Group).

The compositions described herein may be used diluted into a larger volume of water to form a 'working' enzymatic cleaning composition. Such dilutions may vary, such as 1 part of an inventive composition to 10 – 500 parts water, either on a parts by weight/parts by weight ("w/w") basis, or a parts by volume/parts by volume ("v/v") basis. Advantageously, working enzymatic cleaning compositions are formed when the inventive compositions are combined with water in respective w/w or v/v ratios of 1:10-500, preferably 1:20 – 250, still more preferably and particularly advantageously at a 1:28 ratio.

As has been noted previously, it has been surprisingly found that the inventive compositions do not induce significant amounts of corrosion to steels, notwithstanding the large proportion of water in the compositions. This is particularly surprising and of great commercial significance as such steels are frequently used as materials of construction for medical and dental instruments.

Further optional components include, inter alia, fragrances and colouring agents such as dyes and pigments. Other optional constituents known to the related art might also find use in the present inventive compositions.

The invention also features a method of cleaning any rinsable surface or cleaning medical instruments and related equipment by providing the enzyme-containing aqueous composition such as that described herein, and exposing or immersing the surface or instrument to said enzyme-containing aqueous composition for a time sufficient to effect cleaning of the instrument.

The enzyme-containing aqueous compositions may be used in virtually any application where enzymatic cleaning of hard surfaces, particularly of metal surfaces, is desired. Such metal surfaces which are particularly advantageously treated include those which are associated with the provision of health care services. These include surfaces which may be normally encountered (i.e., operating tables, trays, gurneys, instrument surfaces, equipment surfaces, etc.) as well as medical instruments and devices. Exemplary of medical instruments and devices are endoscopes, surgical instruments, operating room handpieces, ventilation tubes, or dental handpieces. Rinsable surfaces include any surface that is, or has been, in contact with organic material such as protein, fats, blood, carbohydrates and similar material.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

Examples

The following examples illustrate aspects of the preparation and performance of the enzyme-containing aqueous cleaning compositions described above. The compositions are formed by simply admixing the constituents to the water, and stirring to ensure homogeneity.

Examples of compositions according to the invention, including those of certain particularly preferred embodiments, are indicated in the following Table 1.

TABLE 1	E1	E2	E3	E4	E5	E6	E7
brass corrosion inhibitor	--	0.1	--	0.1	--	--	--
calcium chloride	0.2	0.2	0.2	0.2	0.2	0.2	0.2
triethanolamine	5.0	5.0	5.0	5.0	5.0	3.0	1.0
boric acid	3.0	3.0	3.0	3.0	3.0	2.0	2.0
propylene glycol	15.0	15.0	15.0	15.0	15.0	15.0	15.0
non-ionic surfactant	6.45	6.45	6.45	6.45	6.45	6.45	6.45
protease enzyme (16.0 KNP units/gram)	3.0	3.0	3.0	3.0	3.0	3.0	3.0
amylase enzyme (300 KN units/gram)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
isopropyl alcohol	--	8.0	8.0	--	5.0	--	--
dye	--	0.004	--	0.004	--	--	--
perfume	--	0.15	--	0.15	--	--	--
water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

The constituents indicated on Table 1 are indicated as %weight of the named constituent. Water was added in 'quantum sufficient' to produce 100%wt. of a composition. These represent 'concentrated' forms of the inventive compositions which are expected to be diluted into a larger volume of water to form a 'working' enzymatic cleaning composition. The constituents used to form these concentrates were used 'as supplied' by the respective supplier. The identity of the specific constituents, and their %weight of actives (when not 100%wt. actives,) are indicated on Table 2, following.

TABLE 2	
brass corrosion inhibitor	COBRATEC 99, 1-H-benzotriazole
calcium chloride	anhydrous calcium chloride
triethanolamine	triethanolamine (100%wt. actives) [Aldrich Scientific Corp.]
boric acid	(100%wt. actives) [Aldrich Scientific Corp.]
propylene glycol	(100%wt. actives) [Aldrich Scientific Corp.]
non-ionic surfactant	PLURONIC L-62 LF (100% actives) [BASF Corp.]
protease enzyme (16.0 KNP units/gram)	Savinase 16.0L
amylase enzyme (300 KN units/gram)	Termamyl 300
isopropyl alcohol	(100%wt. actives) [Aldrich Scientific Corp.]
dye	proprietary composition of its manufacturer/supplier
perfume	proprietary composition of its manufacturer/supplier
water	deionized water

Cleaning Efficacy

The compositions exhibit good cleaning efficacy, particularly on metals having organic soils on their surfaces.

The following test method was used to determine the efficacy of an enzymatic cleaner for the medical industry.

First, a test soil was made from 90%wt. egg yolk and 10%wt. lamb's blood, which were mixed to form a homogenous mixture. This mixture was made within an hour of application to the test (stainless steel) coupons described below. This combination of materials was chosen because the egg yolk and lamb's blood contain the lipids and proteins which exemplify difficult types of body/medical waste to enzymatically clean.

Next, a series of 1 inch (2.5 cm) by 2 inch (5.1 cm) stainless steel coupons, 1/8 inch (0.32 cm) thick with a 1/8 inch (0.32 cm) hang hole at the top, were cleaned and dried. The coupons were dipped in the test soil to cover 90% of the surface of each

coupon. The dipped coupons were then hung to dry at 25°C for 24 hours. After drying, the weights of the soiled coupons were recorded as the initial un-cleaned weight.

Subsequently diluted samples of enzymatic cleaning compositions, including samples based on the inventive compositions were produced and placed into beakers large enough to submerge the soiled section of the stained coupons. These diluted samples were 1:128 w/w dilutions of an enzymatic cleaning composition in a larger volume of deionized or distilled water. The enzymatic cleaning compositions used to form the diluted samples had been formed two months prior to the present test, and had been stored at the various temperatures indicated in Table 3.

The coupons were hung in the samples for a measured time period (20 minutes) and then removed. Upon removal, the coupons were rinsed under 25°C water at a flow rate of 66.67 ml/sec. The coupons were held at approximately a 30° angle to the stream of water, 7.6 cm from the faucet, and passed through the stream 3 times on each side so that the entire coupon had been rinsed. The coupons were then hung to dry for 24 hours at 25°C. After drying, the coupons were weighed and compared to the initial weights for the weight loss due to soil removal.

TABLE 3	
Formulation [storage temperature]	weight loss of soil observed
E3 [4.5°C]	0.075 grams
E3 [25°C]	0.075 grams
E3 [40.5°C]	0.075 grams
E3 [49°C]	0.074 grams
water	0.009 grams

These results show the superior cleaning characteristics of the 1:128 w/w dilution of the composition according to the invention, as compared to the 'control sample' of water which was significantly less effective. Further, the results illustrate the excellent storage stability of the compositions over a wide temperature range (4.5°C - 49°C), and their efficacy subseqent to such storage.

Evaluation of High-Temperature Stability

The temperature stability of a composition according to the invention (E3, per Table I) was evaluated in the following manner.

Samples of the formulation according to E3 were prepared, and then stored for two months at the various temperatures indicated on Table 4, below. Similarly, a sample of a commercially available enzyme containing cleaning composition "ENZOL" (ex. Johnson & Johnson) was also stored and used as a comparative example.

Subsequent to the two-month storage period, the samples were removed from their storage location and were brought to 25°C, and working dilutions were made therefrom by forming 1:128 w/w dilutions of an enzymatic cleaning composition in a larger volume of deionized or distilled water.

Unexposed X-ray film was cut into 1.3 cm wide strips 7.6 cm long. The strips were marked at 1.3 cm intervals and then suspended from a ring stand. All strips were placed equal distance from the bench top. The X-ray film strips were then lowered into the samples to the first 1.3 cm mark. After 2.5 min., the film strips were lowered to the next 1.3 cm mark. This was repeated until the total soak time elapsed reached 12.5 min. at which time the film strips were removed from the samples. After removal, the film strips were immediately placed in clean water. Each film strip was then rinsed with 30°C water by holding the film strip horizontally 7.6 cm below the faucet. The film strip was then passed under the water stream for 6 one second passes. This was done for each side of the film strip. The flow rate of the water was 66.67 ml/sec. The film strips were then allowed to dry.

The activity of the protease in the product was monitored by comparing the results of the high temperature samples to the refrigerated samples results. The product solution would clear the X-ray film in a measured time period. The longer the time it took to clear the film, the weaker the protease in the sample. The first 1.3 cm interval on the film strip to be submersed into the solution would be interval submersed for the total time.

The results of these tests are indicated in Table 4.

TABLE 4		
Sample	Temperature	Time to Clear Film (Minutes)
E3	4.5°C	10.0
E3	25°C	10.0
E3	40.5°C	10.0
E3	49°C	10.0
ENZOL (Lot L080671)	4.5°C	>12.5

As a review of the reported results on Table 4 indicates, the samples according to the invention exhibited excellent storage stability characteristics subsequent to cold as well as hot storage conditions. This is particularly surprising as aqueous enzyme containing compositions are expected to lose a significant proportion of their initial storage stability when stored at elevated temperatures. As can be see, the inventive compositions exhibited nearly identical performance characteristics subsequent to storage over a wide temperature range.

Metals Corrosion Testing

A corrosion test was performed according to the following method:

Working solutions of the inventive composition and two competitor compositions were prepared in accordance with the respective label directions. The dilution rate was 2 parts composition to 128 parts water. Each batch thus prepared was divided into smaller beakers.

Metal coupons of various types were placed in the beakers so that ¾ of the coupon was submerged. The coupons were left at room temperature for 24 hours. The coupons were then removed and rinsed with water and lightly dried with paper towels. The results of this test are indicated in Table 5.

TABLE 5					
	E1	E3	ENZOL	ORTHOZIME	water
metal coupon type					
Steel (SAE-1010CR)	2 small rust dots imperfections in metal	1 small rust dot imperfections in metal	wide spread rust	wide spread rust	wide spread rust
Steel	2 small rust dots imperfections in metal	1 small rust dot on edge, imperfections in metal	wide spread rust	wide spread rust	wide spread rust
Copper	no effect	no effect	no effect	no effect	no effect
Brass CA-260 (ASTM B-36)	black discoloration on 40%	black discoloration on 35%	black discoloration at water line	no effect	light black on 60%
Stainless Steel (Type 316)	no effect	no effect	no effect	no effect	no ffect

ENZOL is an enzyme containing cleaning composition available from Johnson & Johnson

ORTHOZIME is an enzyme containing cleaning composition available from Ruehoff Inc.

The results of the test were determined by visually observing the metal coupons; the results show that the compositions according to E1 and E3 inhibit rusting on steel.

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

CLAIMS

1. An aqueous cleaning composition comprising:

an enzyme system comprising

a protease having an activity of at least about 0.10 Kilo Novo Protease units/gram, and

an amylase having an activity of at least 0.5 Kilo Novo units/gram;

from about 0.2% to about 10% by weight of a boron-containing compound;

from about 10% to about 30% by weight of a polyhydroxy-containing compound;

from about 1% to about 10% by weight of an alkanolamine;

from about 0.01% to about 2.0% by weight of a water-soluble calcium salt;

from about 1% to about 20% by weight of a nonionic surfactant;

from about 3% to about 10% by weight of an alkanol; and

water sufficient to provide the remaining weight of the composition,

wherein the composition has a pH of from about 7.0 to about 8.0.

2. The composition according to claim 1, further comprising a brass corrosion inhibitor.

3. The composition according to claims 1 or 2, further comprising fragrance and colour constituents.

4. A composition according to any of claims 1 to 3 in which the protease is derived from the genus *Bacillus*.

5. A composition according to any of claims 1 to 3 in which the amylase is derived from the genus *Bacillus*.

6. The composition according to any of the preceding claims, wherein the alkanolamine is triethanolamine.

7. The composition according to any of the preceding claims, wherein the nonionic surfactant is a copolymer of alkylene oxides.

8. The composition according to claim 7, wherein the alkylene oxides are selected from ethylene oxides and propylene oxides, and the copolymer is a block copolymer.

9. The composition according to any of the preceding claims, wherein the boron-containing compound is boric acid.

10. The composition according to any of the preceding claims, wherein the polyhydroxy-containing compound is selected from glycerine, ethylene glycol, propylene glycol and mixtures thereof.

11. The composition according to any of the preceding claims, wherein the alkanol is isopropyl alcohol.

12. The composition according to any of the preceding claims which does not contain a corrosion inhibitor to prevent the corrosion of steel.

13. The composition according to any of the preceding claims which retains at least 50% of its enzymatic activity after at least 60 days of storage at temperatures of 40°C.

14. The composition according to claim 13 which retains at least 60% of its enzymatic activity after at least 60 days of storage at temperatures of 40°C.

15. The composition according to any of claims 1 to 11 which does not contain a corrosion inhibitor for preventing the corrosion of steel, and wherein the composition retains at least 50% of its enzymatic activity after at least 60 days of storage at temperatures of at least 40°C.

16. A method of cleaning medical surfaces or instruments, the method comprising exposing a medical surface or instrument to an enzyme-containing cleaning composition according to any of the preceding claims.

17. The method according to claim 15, wherein the medical instrument is selected from the group consisting of endoscopes, surgical instruments, hospital operating room handpieces, ventilation tubes, and dental handpieces.

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Application No: GB 0005840.4 **Examiner:** Michael Conlon
Claims searched: 1-17 **Date of search:** 19 September 2000

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.R): C5D D127 D121

Int Cl (Ed.7): C11D 3/386

Other: Online: WPI EPODOC PAJ

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
A	WO94/29428 A1	(Procter & Gamble) the Examples	1
A	WO93/21299 A1	(Procter & Gamble) Example V	1
A	US5998342	(Scoville)	1
A	US5691292	(Marshall)	1
X	US4537707	(Severson) Example 1	1 at least
A	US4261868	(Hora)	1

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